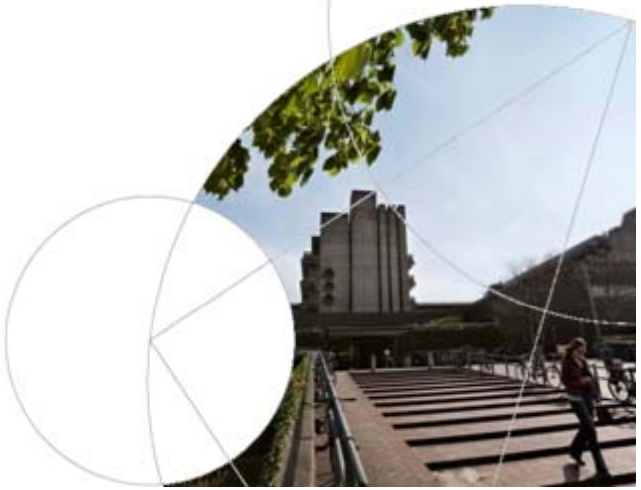
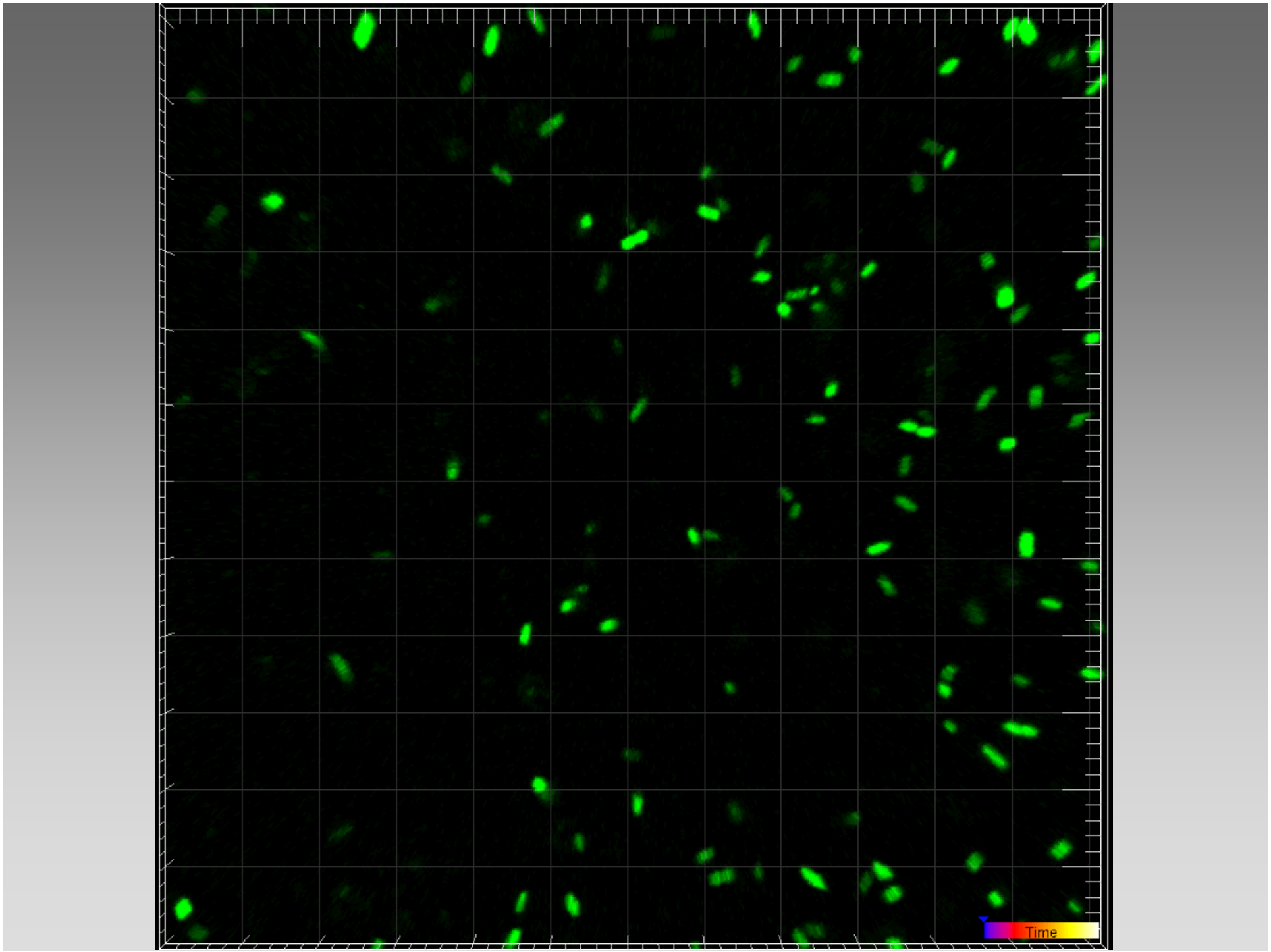
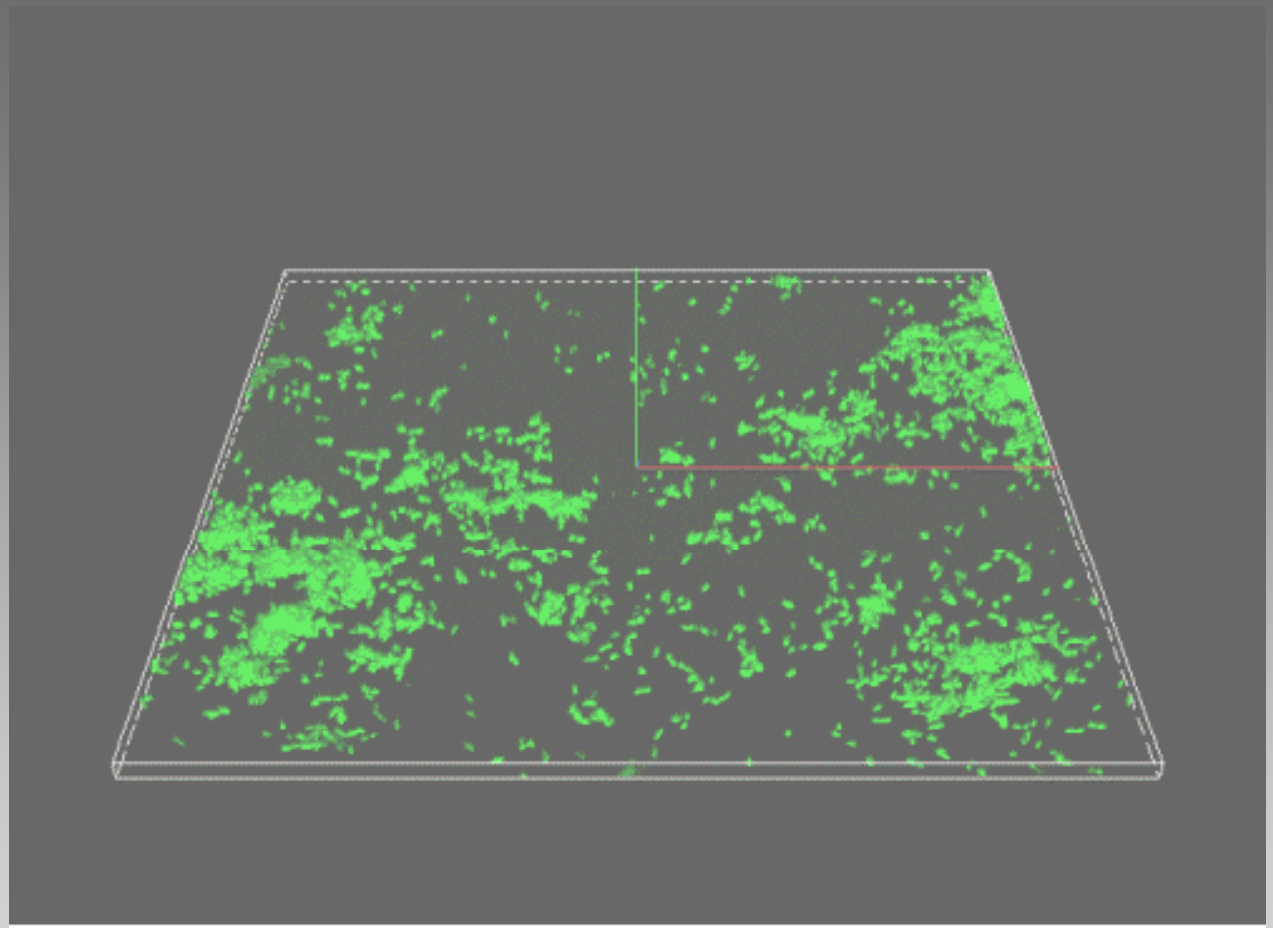
Diagnosis of bacterial biofilm infections

REGION H

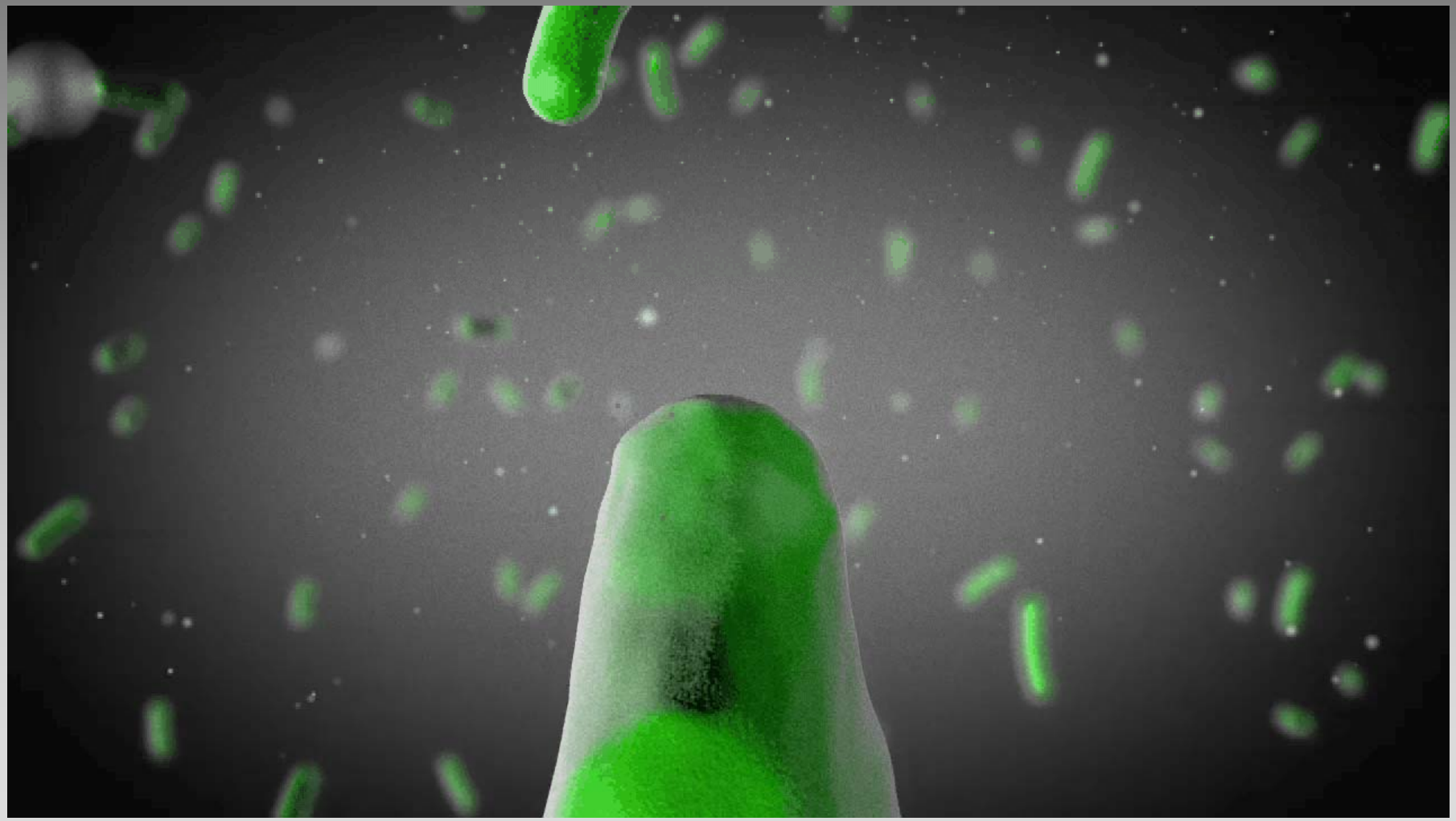
Rigshospitalet



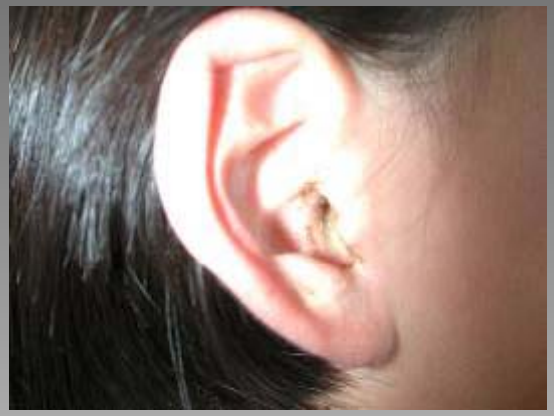

Time

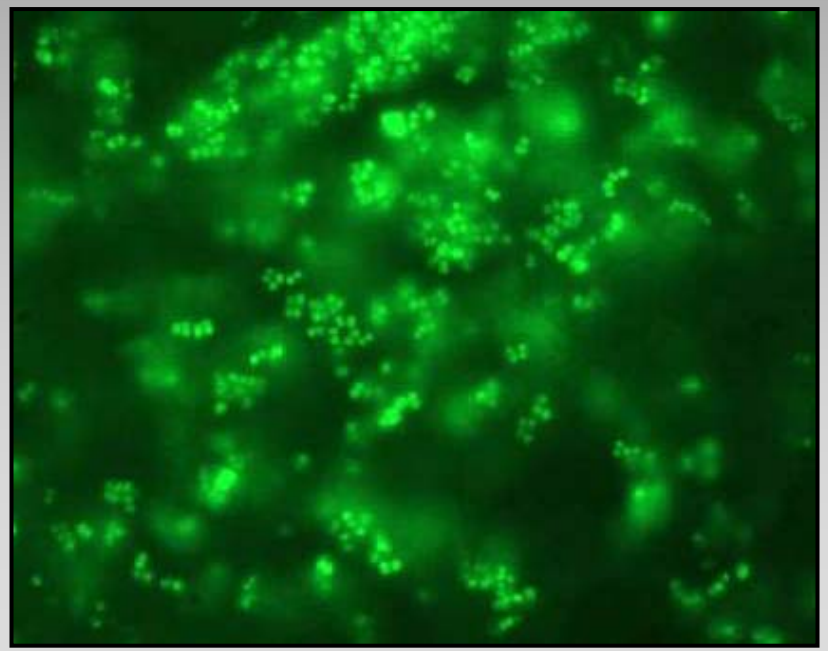


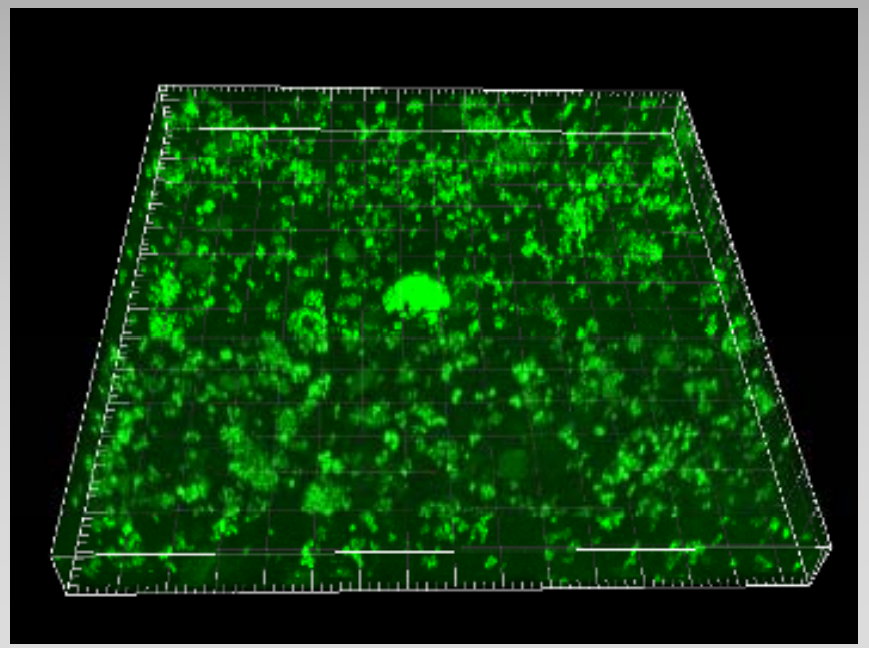
The challenge



Chronic otitis media







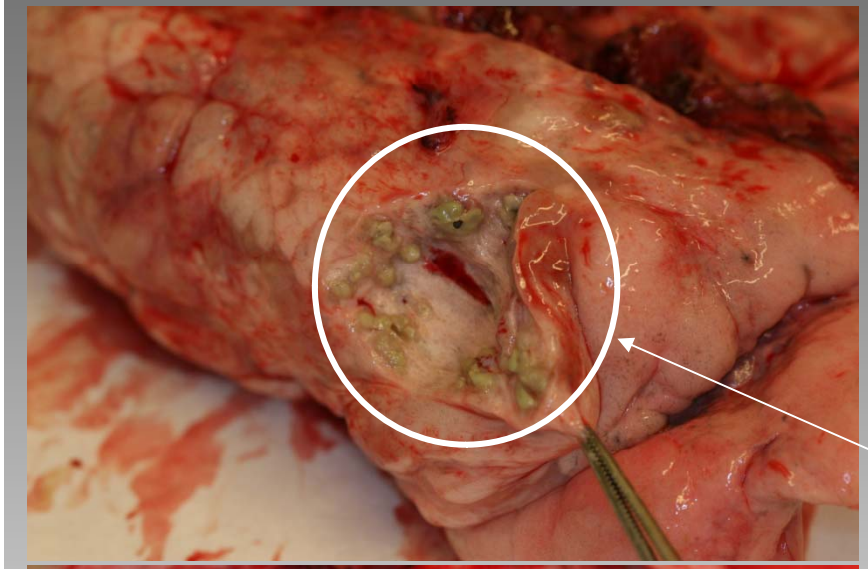
Homøe and Bjarnsholt et al;European Archives of Oto-Rhino-Laryngology: 266(10) 2009, Page 1533

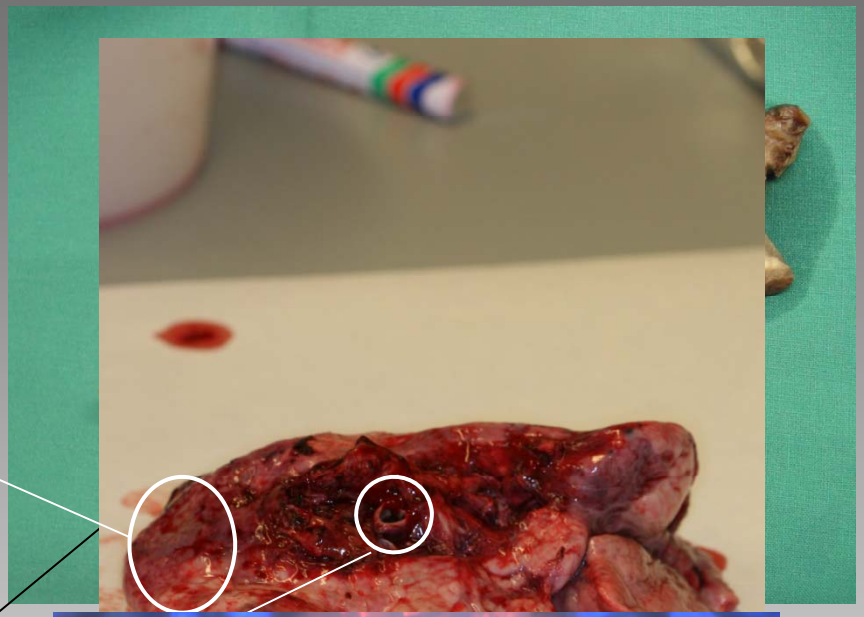
Soft tissue fillers

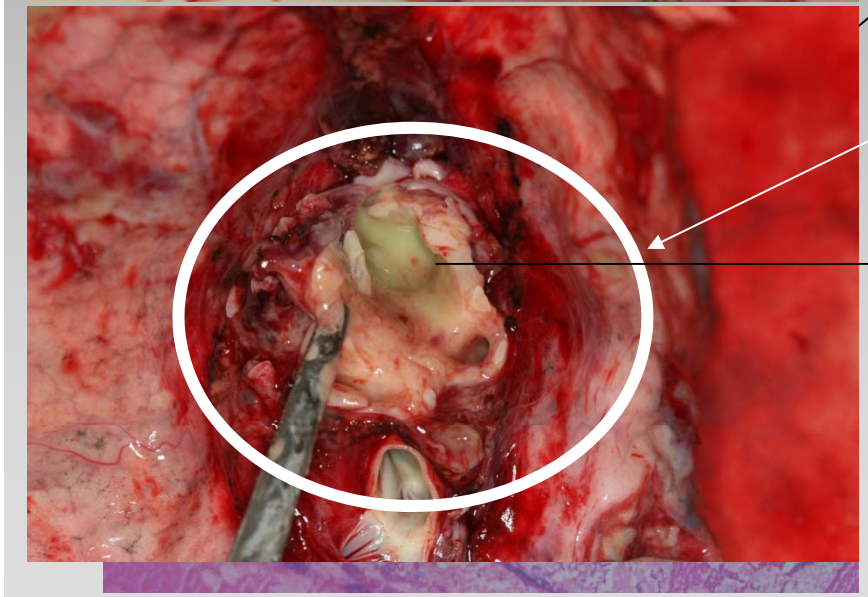
site	Type of PAAG	Time since inj	Initial treatment	Time with AE
Cheek	Aquamid	2 years	Steroid inj Later AB inj	7 months
Lip	Aquamid	½ year	Steroid inj Later ABs	½ year
Lip	Aquamid	1 month	Steroid + ABs Later ABs	2 years
Breast	Amazing gel	2 years	ABs liposuction	5 months
Tear-trough	Aquamid	2 years	Steroid inj Later ABs	½ year
Naso-labial fold	Aquamid	14 days	Steroid ABs+surgery	1½ year
Lip	Interfall gel	½ year	Steroid ABs+surgery	2½ years
Penis	Aquamid	2 years	Steroid AB inj+surgery	1½ years

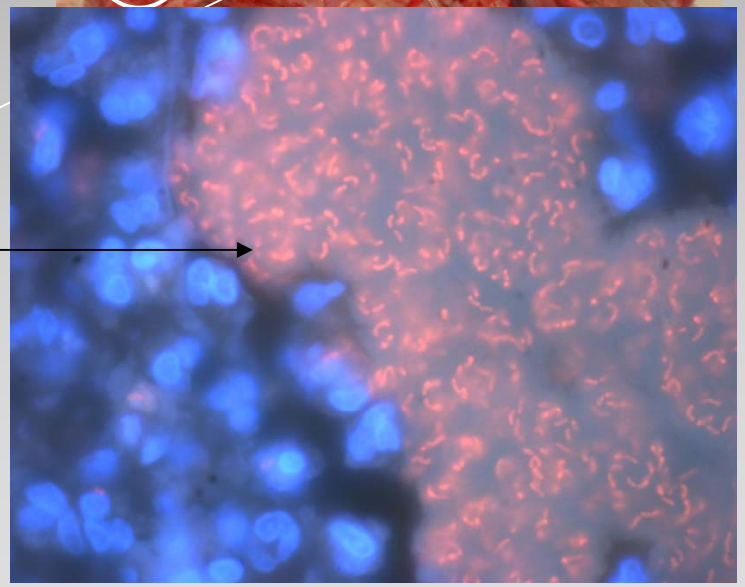
Bjarnsholt et al;; **Dermatol.surg. 2009 (Aug)**

Cystic fibrosis



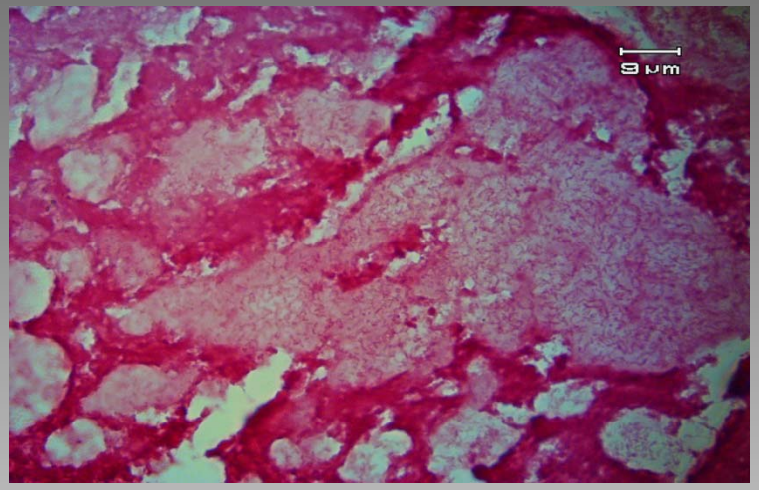


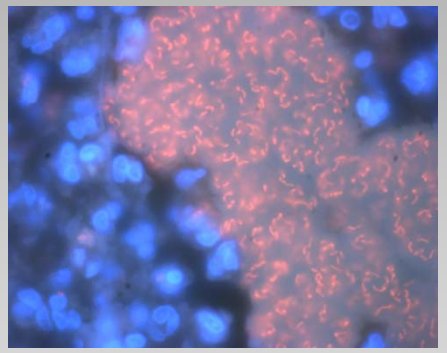


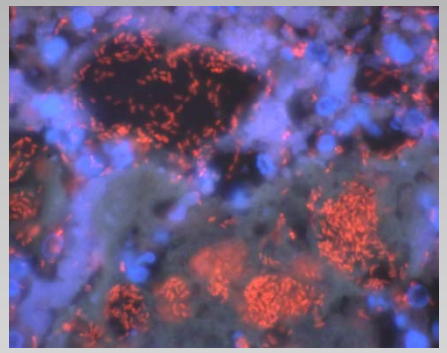


Aggressive suppressive therapy

CF male
28 years of chronic PA infection
2 week anti PA treatments
20 years daily colistin/tobramycin inhalations



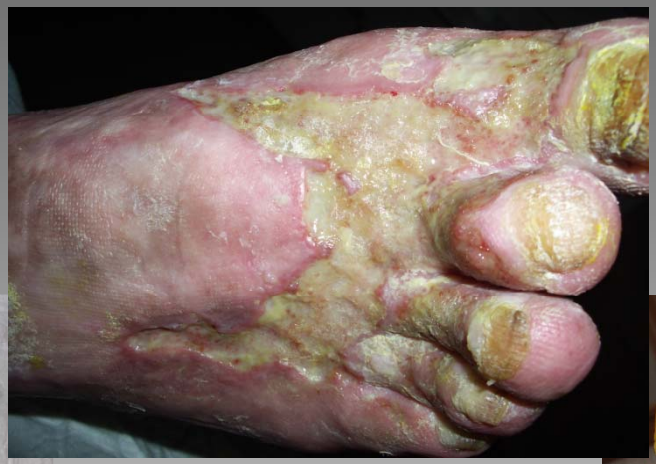


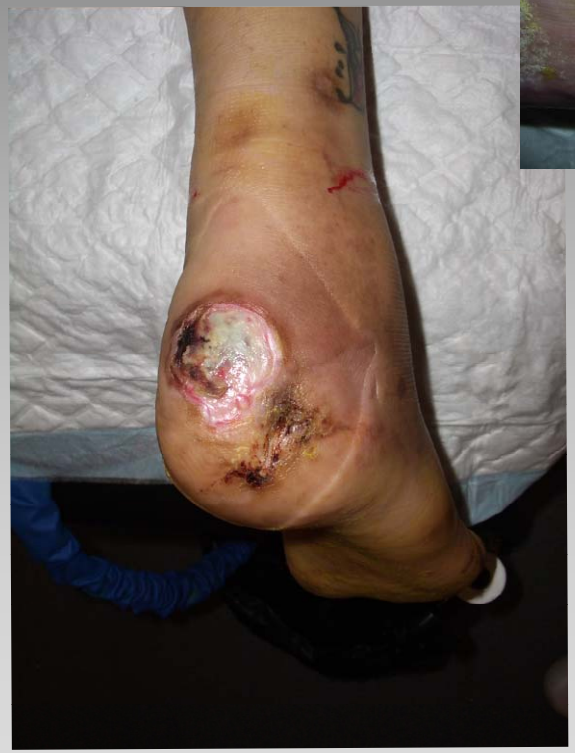


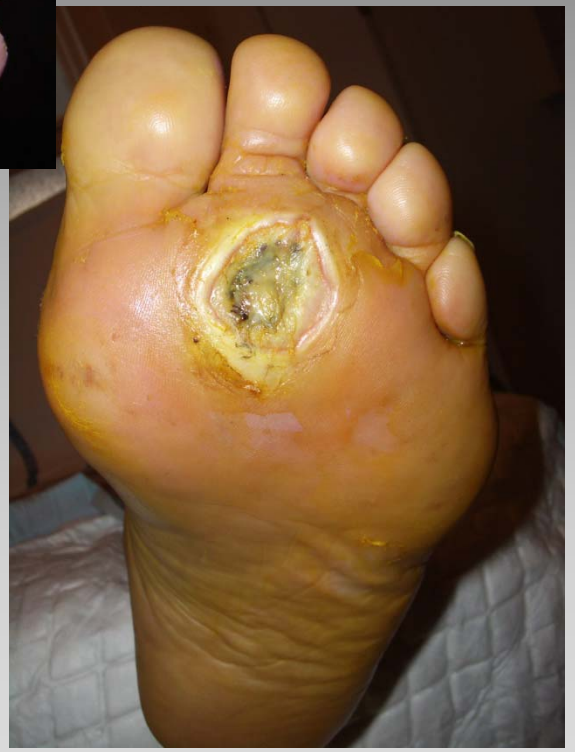
1 kg tobramycin,
10 kg beta-lactam anti-pseudomonas antibiotics
and 1 kg inhaled colistin

Bjarnsholt et al; ***Pseudomonas aeruginosa*** **biofilms in the Respiratory Tract of Cystic Fibrosis Patients**; Pediatr Pulmonol. 2009 Jun;44(6):547-58

Chronic wounds

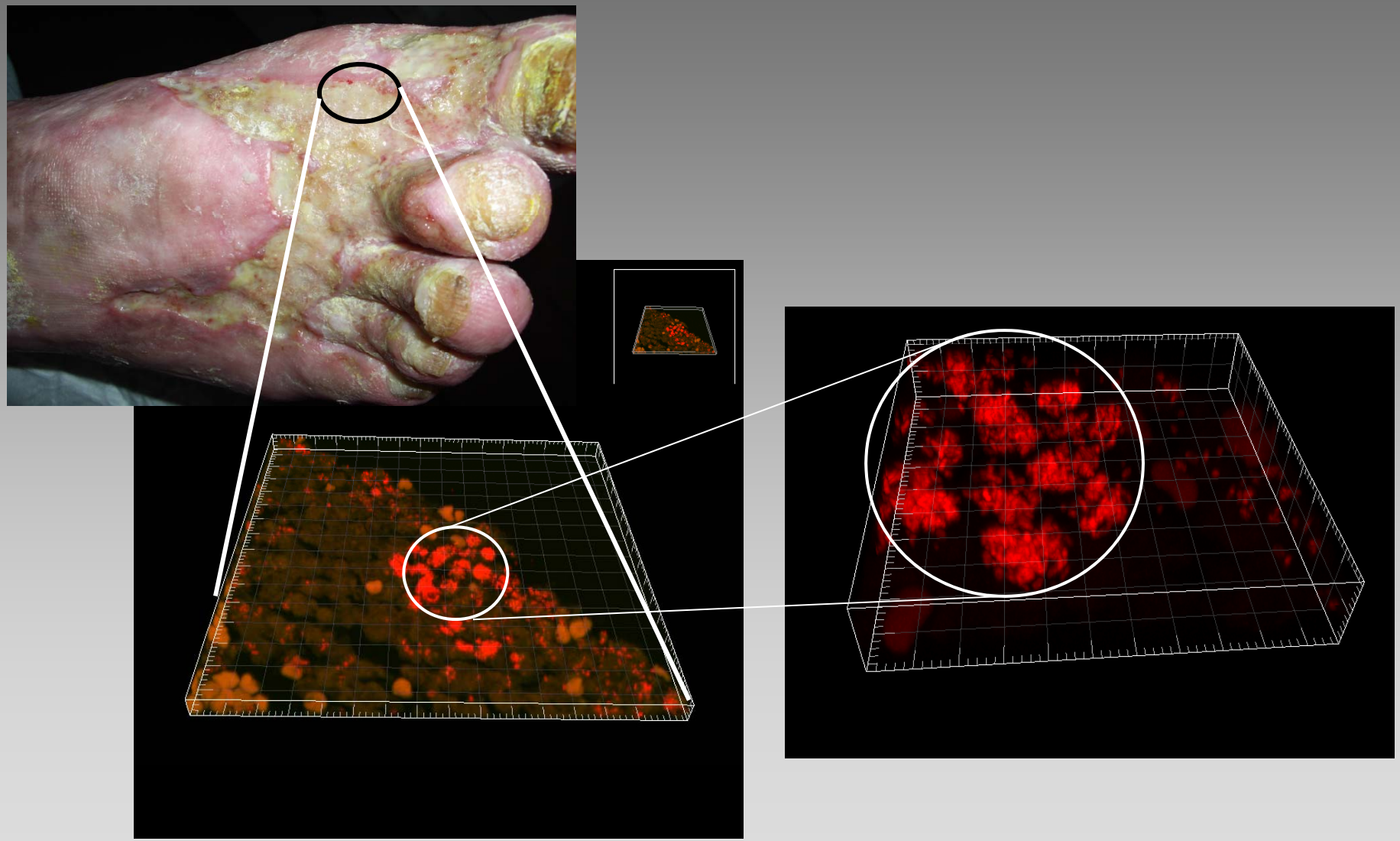






Non-healing despite aggressive treatment

Biofilms in chronic wounds

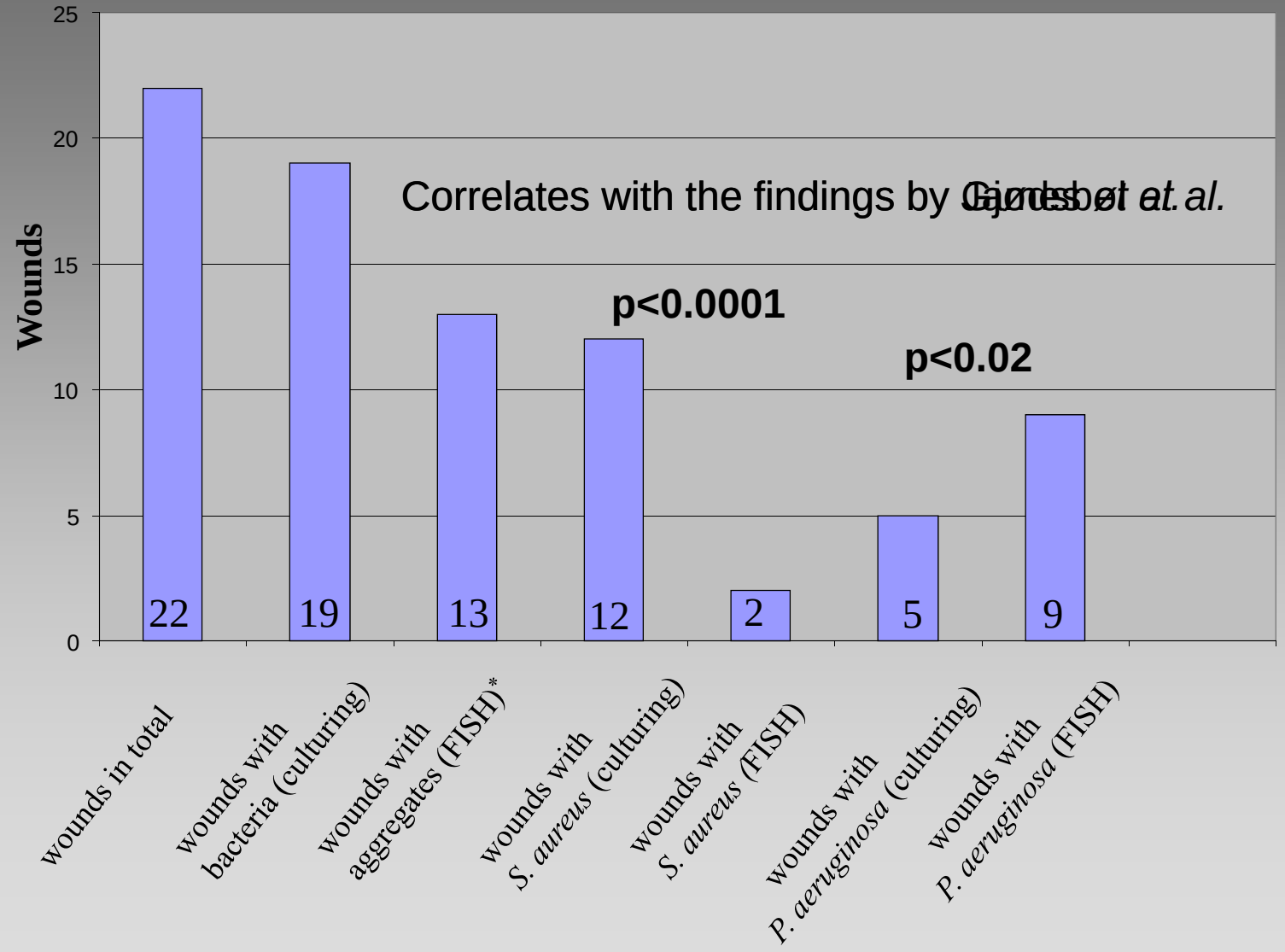


Bjarnsholt et al; Wound Repair and Regeneration, 2008 Jan-Feb;16(1):2-10.

Three aspects of biofilms diagnostics

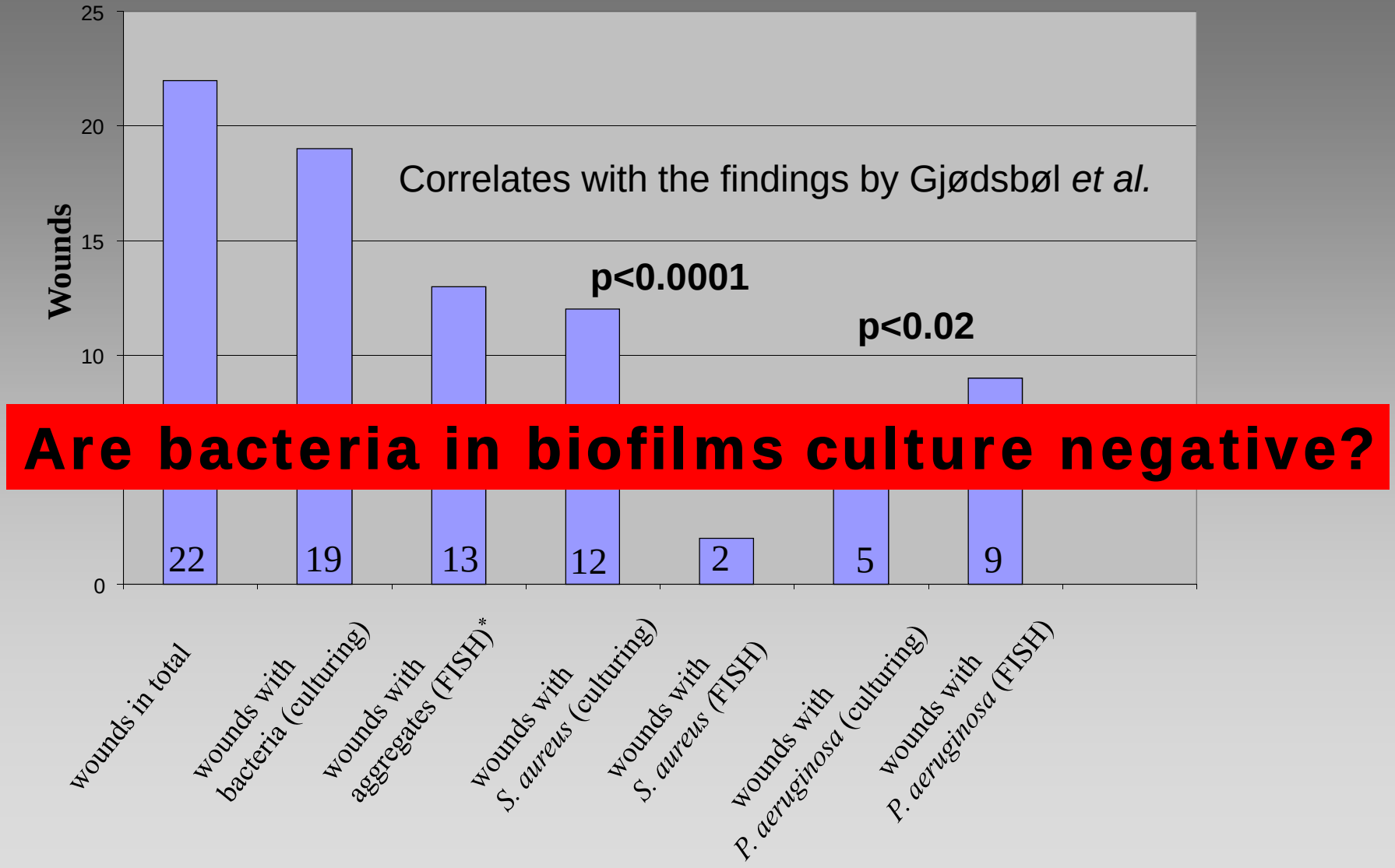
- Sampling
- Identification
- Visualization

Sampling



Kirketerp-Møller…. and Bjarnsholt; The distribution, organization and ecology of bacteria in chronic wounds; J Clin Microbiol. 2008 Aug;46(8):2717-22

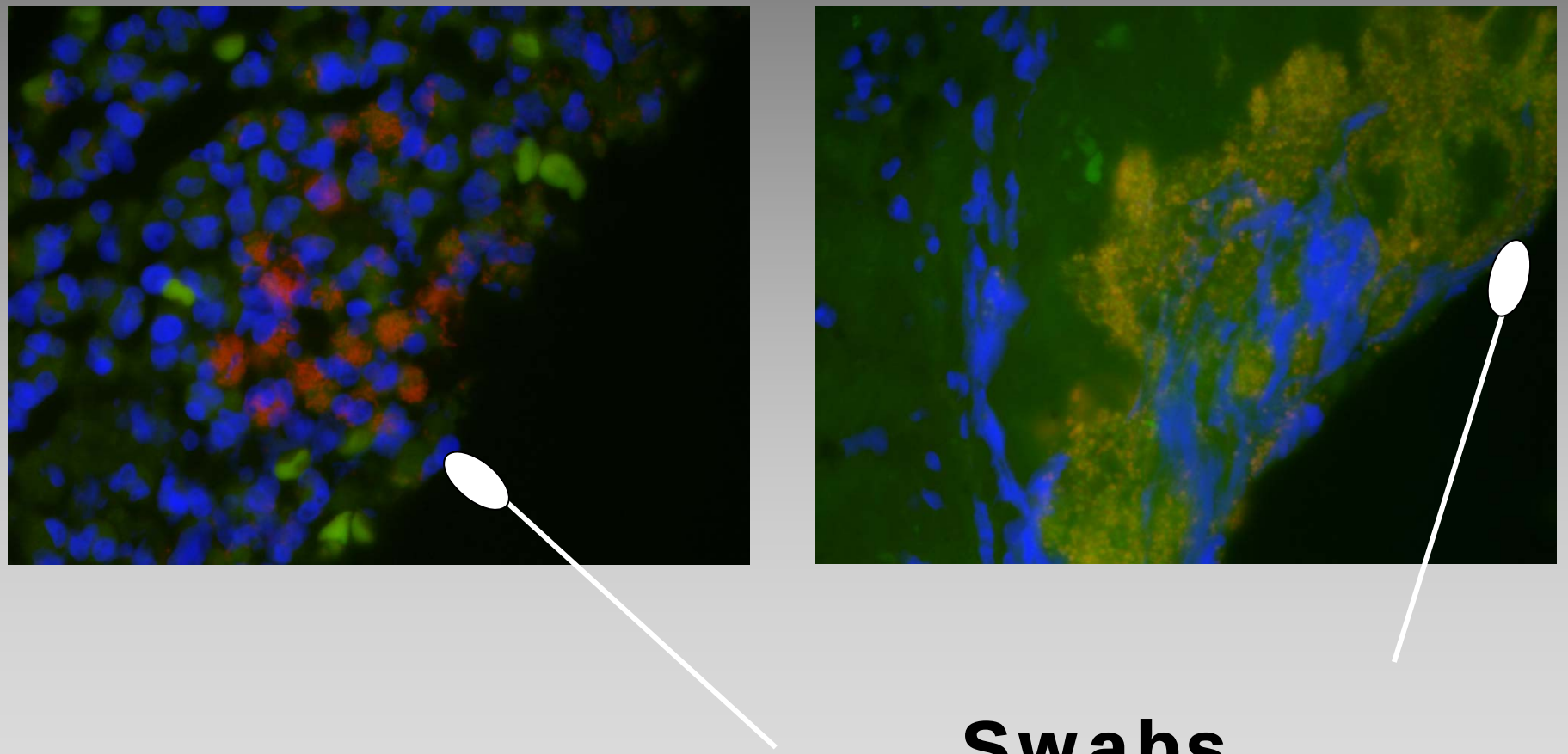
Sampling



Are bacteria in biofilms culture negative?

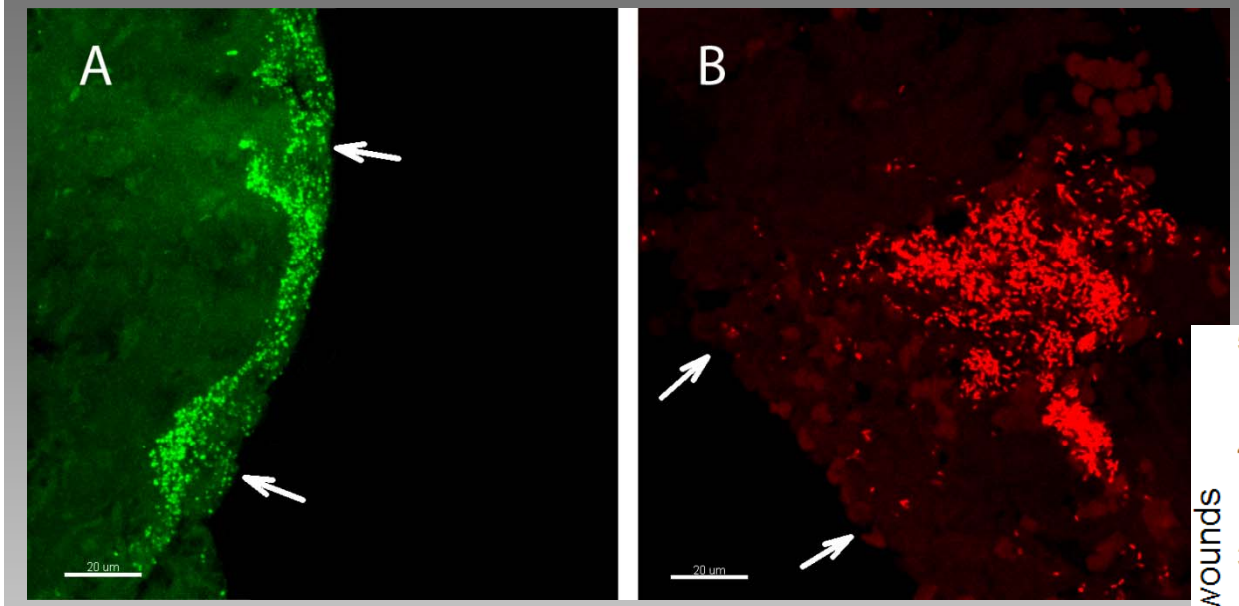
Kirketerp-Møller…. and Bjarnsholt; The distribution, organization and ecology of bacteria in chronic wounds; J Clin Microbiol. 2008 Aug;46(8):2717-22

NO, but they have to be “released” to enable growth

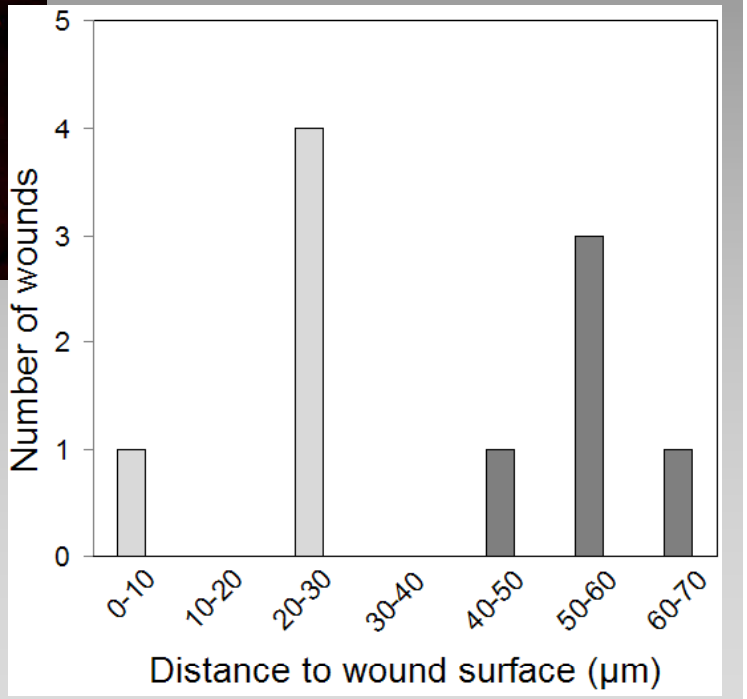


Swabs

Distribution of species



S. aureus *P. aeruginosa*



Fazli and Bjarnsholt et al submitted: J Clin Microbiol. 2009 Dec;47(12):4084-9

***S. aureus* biofilm** □
***P. aeruginosa* biofilm** ■

Sampling

Chronic venous leg ulcer- gene copy numbers of *S. aureus*

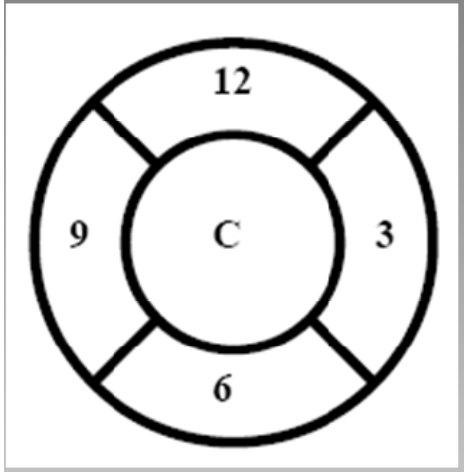
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Previous findings	S, C	S	C	S, C	S, C	S, C	S, C	S, C	S, C	S, C	S, C	S	S, C	C
qPCR findings [no. copies]	138	7	9	8	152	87	10	69	5770	3	nd	67	72	6

Chronic venous leg ulcer- gene copy numbers of *P. aeruginosa*

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Previous findings			C		C		C	C			S, C			
qPCR findings [no. copies]	15	12	18	14	1450	1190	2450	37	34	18	1530	141	43	40

–(Wound Repair and Regeneration 2010 Jan-Feb;18(1):38-49.)

Destribution of bacteria in chronic wounds



	S. aureus			*P. aeruginosa*	
	D	E	F	E	F
Centrum	8	152	87	1450	1190
3	10	21	64	88	557
6	19	89	244	2490	13700
9	21	265	89	127	1760
12	9	493	77	1800	98

The number of gene copies from the investigated microorganisms depend on <u>where</u> in the ulcer the biopsies were collected

–(Wound Repair and Regeneration 2010 Jan-Feb;18(1):38-49.)

Sampling from a surface

- Improved Detection of Biofilm-formative Bacteria by Vortexing and Sonication: A Pilot Study
  - Kobayashi, H. et al; Clin Orthop Relat Res; 2009 May;467(5),
- Sonication is superior to scraping for retrieval of bacteria in biofilm on titanium and steel surfaces in vitro.
  - Bjerkan, G. et al **1:** Acta Orthop. 2009 Apr;80(2):245-50.
- Brief ultrasonication improves detection of biofilm-formative bacteria around a metal implant.
  - Kobayashi, N. et al;Clin Orthop Relat Res. 2007 Apr;457:210-3
- Is the bacteria on the implant or in the tissue?

Summing up:

- Growth might not be the golden standard
  - Biofilm bateria might be hidden
  - Biofilm bacteria might be attached
- However, it is not because bacteria in biofilm can not be cultured
- Combination of growth and different molecular detection methods

Identification -pitfalls

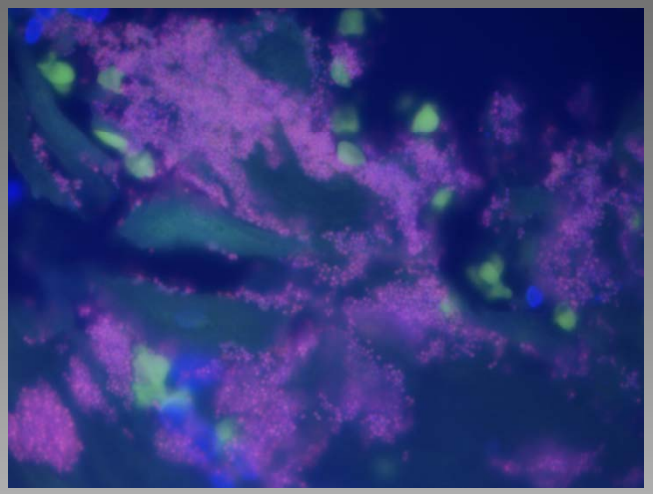
Method	Advantages	Pitfalls and difficulties
Culturing	Bacterial presence is confirmed Antibiotic susceptibility Direct quantification	Heterogeneous distribution Finding the focus Pathogens vs. contamination Biofilms or planktonic samples can be culture-negative
PCR and IBIS	Fast results even when culture is negative Low cut-off	Heterogeneous distribution Finding the focus Pathogens vs. contamination Biofilm or planktonic
Microscopy	Biofilms are confirmed Interactions with tissues Inflammatory cells Results even when culture-negative	Heterogeneous distribution Finding the focus

Identification

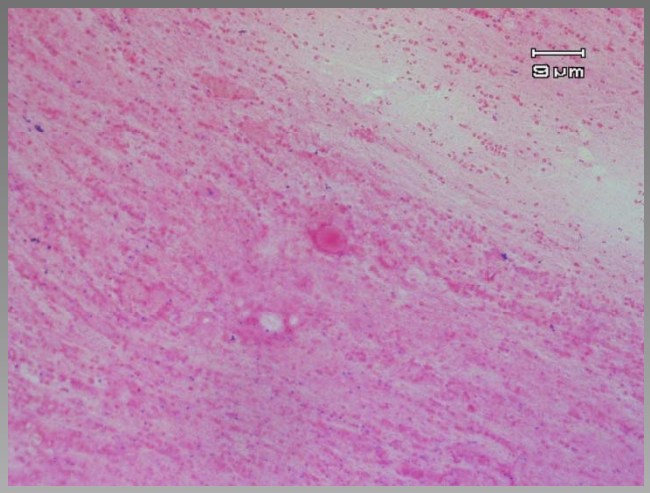
- Average 5.4 species per wound (M)
  - Thomsen…Bjarnsholt et al (WRR submitted 2009)
- Average 6.3 species per wound (C)
  - Gjødsbøl et al (Int. Wound J. 2006)
- Multiple species (M)
  - Dowd et al (PLoS ONE. 2008; 3(10): e3326)
  - James et al (Wound Repair Regen. 2008 Jan-Feb;16(1):37-44)
  - Etc.

(M) = Molecular methods
(C) = Standard culturing

Microscopic ID



Nice and easy



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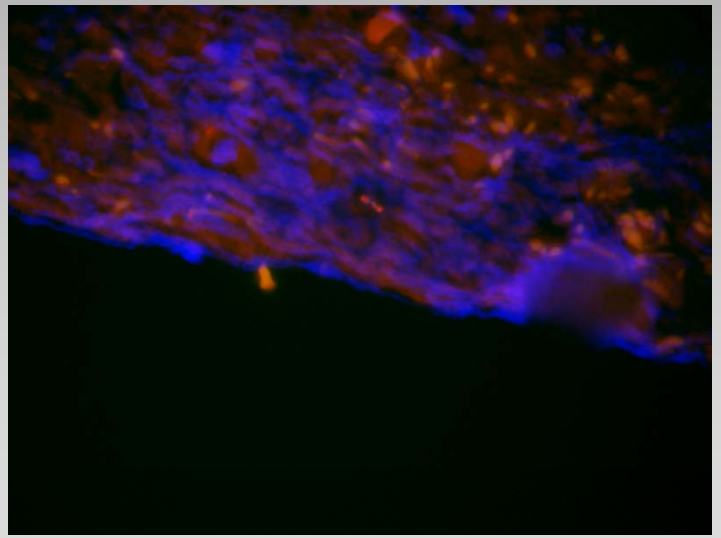

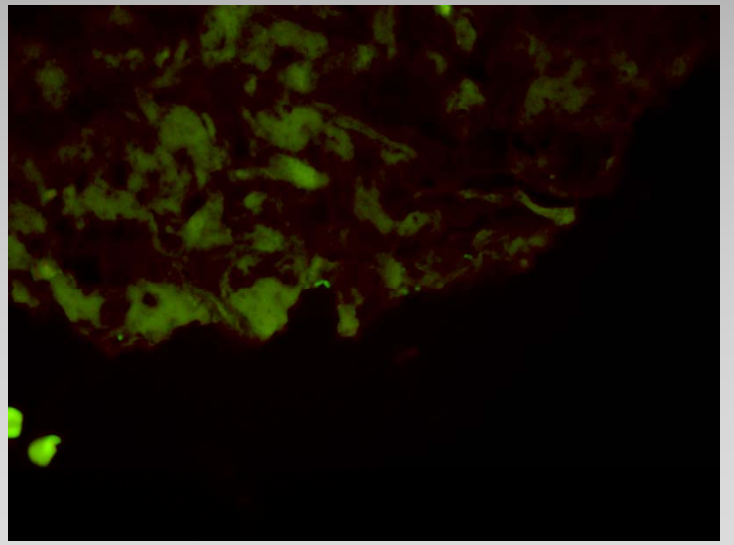

Contamination/planktonic/pathogen/emigrants???

Summing up:

- Both culture and molecular identification reveal on the presence not on orientation/distribution
- 2D or 3D visualization
  - PNA FISH or other species specific staining for direct in situ visualization
- **Sampling**

Summary

- Biofilms are present in most chronic infections
- Bacteria in biofilm are culturable, if released

- Still much to do...

Next steps

- To determine the role, if any, of the numerous bacteria identified in chronic wounds and other chronic infections
  - Are they all contributing to the pathogenesis?
  - Are they all situated as biofilms?
  - Are they all homogenous distributed?
  - Do the anaerobes play a role?
- Inflammatory markers???

<table>
<tr><th colspan="3">Microbiological evidence of localized chronic or foreign body associated infection</th></tr>
<tr><td rowspan="2">Positive culture of a microbe (bacteria, fungus) which is known to cause biofilm infections from one or preferably several or repeated relevant specimens:

• fluid
• swab
• tissue sample</td><td colspan="2">Molecular/non-culture based identification of microbial pathogen</td></tr>
<tr><td>NAT positive results for microbes associated with biofilm infections:

• S. epidermidis or S. aureus with implants,
• P. aeruginosa with CF,
• H. influenzae with COM
• oral streptococci with endocarditis)</td><td>FISH positive results for known biofilm associated microbes showing aggregated microorganisms (in association with microscopic evidence – see microscopic evidence below)</td></tr>
<tr><th colspan="3">Microscopic evidence of aggregated microorganisms</th></tr>
<tr><td>Microscopy revealing the presence of microbial aggregates and biofilm structure (smear or fluid sample, but from tissue sample if possible)</td><td colspan="2">Microscopy revealing evidence of microbial aggregates co-localized with inflammatory cells</td></tr>
</table>

Hall-Stoodley L, Stoodley P, Kathju S, Høiby N, Moser C, William Costerton J, Moter A, **Bjarnsholt T.;**
Towards Diagnostic Guidelines for Biofilm-Associated Infections.;
FEMS Immunol Med Microbiol. 2012 Apr

Medical history of biofilm predisposing condition (implanted medical device, CF, IE, chronic OM)(See Table 1)	
Recurrence of the infection (particularly if evidence is provided that the same organism is responsible at multiple time points)	
Documented evidence/history of antibiotic failure or persistent infection despite adequate choice of antibiotic agent	
Evidence of local or systemic signs and symptoms of infection that resolve with antibiotic therapy, only to recur after therapy has ceased such as:	
Fever, and localised classical signs of infection: • Rubor, redness • Tumor , swelling • Calor, heat • Dolor, pain • Functio laesa , impaired function	Laboratory infection parameters** : • increased white blood cell (WBC) count • CRP, • procalcitonin, • sedimentation ratio • LDH
Evidence of specific immune response to identified microorganism – e.g antibodies to specific pathogens (to alginate or other* P. aeruginosa *antigens in CF patients)	

Hall-Stoodley L, Stoodley P, Kathju S, Høiby N, Moser C, William Costerton J, Moter A, **Bjarnsholt T.;**
Towards Diagnostic Guidelines for Biofilm-Associated Infections.;
FEMS Immunol Med Microbiol. 2012 Apr


Thomas Bjarnsholt
Editor-in-Chief
Claus Moser
Peter Østrup Jensen
Niels Høiby
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NEW ONLINE COURSE IN BIOFILM

In the fall of 2011 the University of Copenhagen is offering a brand new online course on “Bacterial Biofilms and Their Role in Chronic Diseases”. The course can be followed by both Master degree and PhD students, as well as professionals that live up to the admission criteria. For registration and more information, please visit:

www.biofilmcourse.ku.dk

Application deadline is August 2012

Teachers at the course:

Director J. William Costerton
Centre for Genomic Sciences, Allegheny-Singer Research Institute, USA

Assoc Prof Marvin Whiteley
Section of Molecular Genetics and Microbiology, The University of Texas at Austin, USA

Prof Matt Parsek
Department of Microbiology, University of Washington, USA

Director, Phil Stewart,
Chemical and Biological Engineering, Montana State University, USA

Prof, Niels Høiby, MD, Claus Ernst Moser and Dr, Peter Østrup Jensen
Department of Clinial Microbiology, Copenhagen University Hospital, Denmark

Luanne Hall-Stoodley
Southampton Wellcome Trust Clinical Research Facility and University of Southampton Respiratory BRU, Southampton General Hospital, UK

Prof Michael Givskov, Assoc Prof Tim Tolker-Nielsen and Assoc Prof, Thomas Bjarnsholt
Faculty of Health Sciences, University of Copenhagen, Denmark

Prof, Søren Molin
Department for Systems Biology, Technical University of Denmark